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13. ABSTRACT *(Maximum 200 Words)*

We hypothesized that genetic variant of the luteinizing hormone β-subunit, characterized by two point mutations in codons 8 and 15, may increase risk of breast cancer. To test this hypothesis, we conducted a case-control study nested within a prospective cohort that included 270 breast cancer cases diagnosed at age 50 or older and 150 cases diagnosed before age 50. Two controls per case were randomly selected among cohort members who were alive and free of disease and who matched the case on menopausal status, age, date of enrollment, and phase and day of menstrual cycle for premenopausal women. Presence of the variant LH status (heterozygotes plus homozygotes) was not associated with an apparent increase in breast cancer risk in postmenopausal women (OR = 1.07, 95% CI = 0.68 - 1.69). The risk estimate did not change after adjustment for potential confounders (OR = 1.11; 95% CI = 0.69-1.78). In premenopausal women, adjusted risk estimate was (OR = 1.68, 95% CI = 0.89 – 3.17). The results do not appear to support the hypothesis that the variant LH is associated with a significantly increased risk of breast cancer, although they suggest a moderate increase of breast cancer risk among younger women.

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INTRODUCTION

An immunological variant of luteinizing hormone (LH), characterized by two point mutations in codons 8 (TGG→CGG) and 15 (ATC→ACC) of the LH β-subunit gene and an increased bioactivity, has been recently identified by investigators at the University of Turku, Finland. The purpose of this research was to examine the risk of breast cancer associated with the presence of the variant LH, separately in premenopausal and postmenopausal women, and to examine the relationship between the presence of variant LH and serum levels of major steroid hormones among breast cancer and controls in a large NCI-sponsored on-going prospective cohort study of hormones and breast cancer – the NYU women's Health Study.

BODY

Technical Objective 1: Association between LH variant status and breast cancer.

Task 1: Month 1: Preparation of the study procedures and protocols has been achieved, including batch size definition, quality controls preparation, and sample shipments. Meeting of the PI with Dr. Ilpo Huhtaniemi, co-investigator from University of Turku, Finland. Dr. Huhtaniemi gave a presentation in the NYU School of Medicine, describing the discovery and biological properties of the variant LH.

Task 2: Months 2-4: Preliminary assessment of variant LH prevalence and reproducibility of measurements of variant and wild-type LH in frozen serum from the same individuals was achieved. The results of the preliminary study using 40 subjects (two samples each: one never defrosted and one previously defrosted) had shown that variant LH is a common polymorphism with 13% prevalence in the NYU Women's Health Study cohort population. Preliminary study has also shown an excellent reproducibility of the results of variant LH measurements between never defrosted and previously defrosted serum samples (Pearson $r = 0.99$).

Task 3: Months 5-11: Computer database was prepared. Samples of all breast cancer cases identified to date were retrieved from the storage, allocated to appropriate batches and verified. Sample identifiers were deleted and prepared samples were shipped to the laboratory in Turku, Finland in dry ice.

Task 4: Months 6-15: Laboratory analyses in Turku, Finland were performed. Laboratory data was transferred to the computer database and verified. Database was updated with additional information on lifestyle and anthropometric characteristics, reproductive experience, and medical history available from the parent NYU Women's Health Study.

Task 5: Months 16-20: Statistical analyses of the results of Technical Objective 1 were performed. Interim and final manuscripts were written (see Reportable Outcomes section).

Technical Objective 2: Relationship between LH variant status and serum levels of ovarian steroid hormones.

Task 6: Months 8-22: Laboratory analyses of steroid hormones by the NYUWHS.

Task 7: Months 10-24: Creation of hormone result database.

Task 8: Statistical analyses of the results and report writing.

This section of the study has to be delayed because the results of the pertinent laboratory analyses of serum for steroid hormones (testosterone, androstenedione, estrone, estradiol, bioavailable estradiol) in the parent NYU Women's Health Study are not yet available. It is expected that these analyses will be completed by December 2000. This objective will be completed as soon as steroid hormone analyses are performed in the parent study.

KEY RESEARCH ACCOMPLISHMENTS

Analysis of postmenopausal women

- A total of 270 postmenopausal breast cancer cases diagnosed at age 50 or older (229 invasive and 41 non-invasive) and 540 matching control subjects were included in the analysis.
- Out of 810 subjects included in the analysis, 89 had low assay 1/assay 2 LH ratio (83 heterozygous and 6 homozygous subjects) corresponding to the variant LH prevalence rate of 11.0% in postmenopausal group. There was no significant difference in the frequency of LH variant between breast cancer cases and controls (11.5% versus 10.7%, respectively, p = 0.75).
- Presence of the variant LH status (heterozygotes plus homozygotes) was not associated with an apparent increase in breast cancer risk (OR = 1.07, 95% CI = 0.68 - 1.69).
- Adjustment for height, Quetelet's index, age at menarche, age at first full-term pregnancy, history of a prior benign breast condition and first-degree family history of breast cancer did not notably change the risk estimate (OR = 1.11; 95% CI = 0.69-1.78).
- Because frequency of the variant LH status varies with ethnicity, we restricted analyses to Caucasian subjects, the most common ethnic group in the study, but the risk estimate remained close to unity (OR = 0.97; 95% CI = 0.53-1.77).
- The results do not appear to support the hypothesis that the variant form of LH is associated with an altered risk of breast cancer among postmenopausal breast cancer cases and controls.
- It is conceivable that higher bioactivity of the variant LH coupled with its shorter half-life could compensate for each other with no apparent effect on breast cancer risk.

Analysis of premenopausal women

- A total of 150 cases of breast cancer (123 invasive, 22 *in situ*) diagnosed before age 50 and 299 matching control subjects were included in the analysis.

- Out of 449 subjects included in the analysis, 72 had low assay 1/assay 2 LH ratio (69 heterozygous and 3 homozygous subjects) corresponding to a variant LH frequency of 16% in premenopausal group. Breast cancer cases had higher frequency of LH variant as compared to controls (18.6% versus 14.7%) but this difference was not statistically significant ($p = 0.29$).
- Variant LH status (heterozygotes plus homozygotes) controlled only for matching factors was associated with a non-significant increase in breast cancer risk (OR = 1.34, 95% CI = 0.79 – 2.26).
- Adjustment for height, Quetelet index, age at menarche, parity, history of a prior benign breast condition and first-degree family history of breast cancer resulted in a higher risk estimate (OR = 1.68, 95% CI = 0.89 – 3.17). However, 95% confidence intervals included unity.
- The results do not appear to support the hypothesis that the variant form of LH is associated with a significantly increased risk of breast cancer, although they suggest a moderate increase of breast cancer risk among younger women.

REPORTABLE OUTCOMES:

Manuscripts:

1. Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Pettersson K, Huhtaniemi I. Genetic variant of luteinizing hormone and risk of breast cancer in older women. *Cancer Epidemiol Biomarkers Prev* 2000; **9:** 839-842.
2. Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Pettersson K, Huhtaniemi I. Luteinizing hormone -subunit variant and risk of breast cancer in women before age 50. *Cancer Epidemiol Biomarkers Prev* (submitted for publication).

Abstracts:

3. Toniolo P, Akhmedkhanov A, Zeleniuch-Jacquotte A, Pettersson K, Huhtaniemi I. Genetic variant of luteinizing hormone and risk of breast cancer. Proceedings of the "Era of Hope" Department of Defense Breast Cancer Research Program Meeting, Atlanta, GA, June 8-11, 2000, P. 473.

Presentations:

4. Toniolo P, Akhmedkhanov A. "Genetic variant of luteinizing hormone and risk of breast cancer", "Era of Hope" Department of Defense Breast Cancer Research Program Meeting, Atlanta, GA, June 8-11, 2000.

CONCLUSIONS:

- We conclude that variant LH is not associated with an altered risk of breast cancer among postmenopausal women.

- The results do not appear to support the hypothesis that the variant form of LH is associated with a significantly increased risk of breast cancer, although they suggest a moderate increase of breast cancer risk among younger women.

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1. Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Pettersson K, Huhtaniemi I. Genetic variant of luteinizing hormone and risk of breast cancer in older women. *Cancer Epidemiol Biomarkers Prev* 2000; **9:** 839-842.
2. Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Pettersson K, Huhtaniemi I. Luteinizing hormone -subunit variant and risk of breast cancer in women before age 50. *Cancer Epidemiol Biomarkers Prev* (submitted for publication).

APPENDICES:

Attached is the reprint of the published manuscript:

Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Pettersson K, Huhtaniemi I. Genetic variant of luteinizing hormone and risk of breast cancer in older women. *Cancer Epidemiol Biomarkers Prev* 2000; **9:** 839-842.

Appendix 1

1. Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Pettersson K, Huhtaniemi I. Genetic variant of luteinizing hormone and risk of breast cancer in older women. *Cancer Epidemiol Biomarkers Prev* 2000; **9:** 839-842.

Short Communication

Genetic Variant of Luteinizing Hormone and Risk of Breast Cancer in Older Women[1]

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Abstract

A genetic variant of luteinizing hormone (LH) characterized by two point mutations in codons 8 (TGG→CGG) and 15 (ATC→ACC) of the LH β-subunit gene has been described recently. As compared with wild-type LH, the variant LH appears to have higher *in vitro* bioactivity but a shortened circulatory half-life, and it has been reported to affect circulating levels of sex hormones. Our purpose was to determine whether the variant form of LH is associated with an altered risk of breast cancer. This hypothesis was addressed in a case-control study nested within a prospective cohort that included 270 cases of breast cancer and twice as many matching control subjects. The study was limited to subjects diagnosed at age 50 years or older. The LH status was determined by the combination of two immunofluorometric assays of serum using monoclonal antibodies. Frequency of the variant LH was similar in breast cancer cases and controls (11.5% *versus* 10.7%). In conditional regression models, the presence of the variant LH was not associated with a considerable increase of breast cancer risk (odds ratio, 1.07; 95% confidence interval, 0.68–1.69). Adjustment for potential confounders did not notably change the risk estimate (odds ratio, 1.11; 95% confidence interval, 0.69–1.78). These observations do not appear to support the hypothesis that this particular variant of LH is associated with altered risk of breast cancer diagnosed at age 50 years and older.

Introduction

Recently, an immunologically anomalous form of LH[3] has been described in a healthy Finnish woman (1) and was subsequently described in Japan (2). Nucleotide sequencing revealed two missense point mutations in the gene of the LH β-subunit on chromosome 19. One of them (codon 8, TGG→CGG) changes tryptophan to arginine, and the other (codon 15, ATC→ACC) changes isoleucine to threonine (2, 3). Studies of worldwide occurrence of this variant LH revealed a broad variation in frequency from 55.5% in aboriginal Australians to 0% in Kotas from South India (4, 5). The frequency of the variant LH appears to be higher in Scandinavian countries (20–42%), intermediate in Western Europe (15%) and Asia (12–14%), and lower in the Hispanic population in the United States (7%), indicating considerable geographic and racial variation (4).

Analyses of the biological properties of the variant LH suggest that the described mutations may alter the physiological function of LH. The mutation at codon 15 is of particular interest because it introduces an additional glycosylation site to the LH β-subunit, with the potential of altered circulatory half-life and bioactivity (6, 7). *In vitro* studies have shown that the variant LH has elevated bioactivity in homozygous subjects compared with those homozygous for wild-type LH (8, 9), whereas the *in vivo* half-life of the variant LH in circulation was shorter than that for wild-type LH (8). In addition, women heterozygous for the variant LH have somewhat higher serum levels of estradiol, testosterone, and sex hormone binding globulin than women without the variant, indicating alterations in the bioactivity of the variant LH (10). These findings prompted the suggestion of a more potent form of LH with a shorter life span.

Several groups reported that the variant LH is associated with polycystic ovary syndrome (10–12), characterized by increased levels of circulating LH, increased ovarian androgen production, hyperinsulinemia, and multiple cysts in the ovaries, as a result of arrested follicular development. The variant LH may contribute to infertility (2), premature ovarian failure (13, 14), and slow progression of puberty (15). Because LH is an important regulator of steroidogenesis, we hypothesized that the variant form of LH may affect the levels of endogenous sex hormones and the subsequent risk of hormone-dependent cancers. A positive association between endogenous estrogens and breast cancer risk in postmenopausal women was observed in our study population (16) as well as in another prospective cohort (17).

The aim of this study was to determine whether the variant LH is associated with breast cancer in a prospective cohort of mostly Caucasian women from New York City. The present report was concerned exclusively with the risk of cancer diagnosed at or after menopause. Because information about menopausal status at diagnosis was not available for all subjects, we used age as a proxy of menopausal status at diagnosis. Therefore, subjects diagnosed before age 50 years, the median age of menopause at enrollment in the NYU Women's Health Study cohort, were excluded.

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[3] The abbreviations used are: LH, luteinizing hormone; mAb, monoclonal antibody; OR, odds ratio; CI, confidence interval; NYU, New York University.

Materials and Methods

Study Population. Between March 1985 and June 1991, the NYU Women's Health Study enrolled a cohort of 14,275 healthy women, ages 34–65 years, at a breast cancer screening center in New York City. Details concerning subject recruitment have been published elsewhere (16, 18). Women who in the preceding 6 months had neither used hormonal medications nor been pregnant were eligible for enrollment. Blood was drawn before breast examination, between 9 a.m. and 3 p.m., in nonfasting subjects. After centrifugation, serum was divided in 1-ml aliquots and stored at −80°C for subsequent biochemical analyses. Written informed consent was obtained from all cohort members. The study was reviewed and approved annually by the Institutional Board of Research Associates of the NYU School of Medicine.

Nested Case-Control Study. Breast cancer cases were identified primarily by active follow-up either at annual mammographic screening (up to 1991) or through questionnaires mailed to each cohort member every 2–3 years; by computer linkages with Tumor Registries of the states of New York, New Jersey, Connecticut and Florida; by linkages with the United States National Death Index to identify cancer-related deaths; and by review of medical records. Assessment of the quality of follow-up, including capture-recapture analyses within the NYU Women's Health Study, had shown a high rate (95%) of detection of cancer cases (19). By January 1995, of the initial cohort of 14,275 women, 113 (0.8%) were lost to follow-up, and 180 (1.3%) had withdrawn their collaboration. As of January 1, 1995, after 109,111 person-years of follow-up, a total of 417 cases of breast cancer had been identified and confirmed by review of individual clinical and pathological records. Of these, 270 women were 50 years of age or older at the time of diagnosis and were included in the present nested case-control study. For each case, two controls were selected at random from among cohort members who were alive and free of disease at the time of diagnosis of the case and who matched the case on age at entry (± 6 months), date of enrollment (±3 months), and number and dates of subsequent blood donations at the screening clinic.

Laboratory Methods. Because DNA material was not readily available for all subjects, we used a common indirect method using serum to determine the variant LH status. Serum samples from each case and her matched controls were analyzed in the same batch by a laboratory technician who was unaware of their disease status. The LH phenotypes were determined using two different immunofluorometric assays for serum LH determination (DELFIA, Wallac, Finland). The assays used different combinations of mAbs. In the first assay, which recognizes wild-type LH only, the capture mAb recognizes an epitope in the intact α/β-dimer, and the detection mAb recognizes the α-subunit (1). In the second assay, which recognizes both wild-type and variant LH (reference method), two LHβ-specific mAbs were used (20). The ratios of the LH levels measured by these two assays (assay 1:assay 2) fell into three separate categories indicating the LH genotype: (*a*) ≥1.0 (normal ratio), the subject has two normal LHβ alleles; (*b*) 0.15–0.99 (low ratio), the subject is heterozygous for the mutant LHβ gene; and (*c*) 0–0.14 (zero ratio), the subject is homozygous for the variant LHβ gene (8, 11). The intra- and interassay coefficients of variation of assays 1 and 2 were less than 4% and 5%, respectively, at LH concentrations at and above the lowest standard concentration of 0.6 IU/liter of the WHO International Reference Preparation 80/552. Comparison of LH status determination by the immunofluorometric assay and DNA hybridization assay showed identical results regarding the variant LH, and either method can be used as an alternative to determine the LH status (5).

Table 1 Selected characteristics of breast cancer cases and controls, NYU Women's Health Study[a]

Characteristic	Breast cancer cases (n = 270)	Controls (n = 540)	P^b
Age at blood donation (yrs)	58 (44–68)	58 (43–68)	0.86
Age at menarche (yrs)	12 (9–17)	13 (8–17)	0.09
Ever pregnant (%)	79.8	83.1	0.28
Age at first full-term pregnancy (yrs)	26 (16–41)	25 (16–43)	0.04
Breast cancer in first-degree relative (%)	21.9	22.0	0.95
Prior benign breast condition (%)	62.4	51.2	0.005
Height (cm)	162.4 (150–183)	161.6 (145–183)	0.10
Weight (kg)	69 (47–123)	67 (45–141)	0.06
Quetelet index (kg/m^2)	25.9 (17.0–43.5)	25.5 (17.0–54.8)	0.24

[a] Values represent means (range), unless otherwise specified.
[b] χ^2 test for proportions and mixed-effects regression test for continuous variables.

Statistical Methods. Mixed-effects regression models were used to test for differences in continuous variables between case and control subjects, taking into account the matched design (21). For categorical variables, the χ^2 test was used. All reported Ps are two-sided, and Ps less than 0.05 are considered statistically significant.

Conditional univariate and multivariate logistic regression models were used to assess the association between LH status and breast cancer. Potentially confounding variables were included in multivariate logistic models. They included height, weight, Quetelet index (weight in kilograms divided by height in meters squared), age at menarche, age at first full-term pregnancy, history of a prior benign breast condition (positive *versus* negative), and first-degree family history of breast cancer (positive *versus* negative). All variables (except history of a prior benign breast condition and family history of breast cancer) were analyzed as both continuous variables and by quartiles. All analyses were carried out using SAS Version 6.12 software. Results are expressed as ORs and 95% CIs.

Results

A total of 270 postmenopausal breast cancer cases diagnosed at age 50 years or older (229 invasive and 41 noninvasive carcinomas) and 540 matching control subjects were included in the analysis. Some characteristics of the study group are given in Table 1. The majority of study subjects were Caucasian (72%); 8% were African American, 3% were Hispanic, 2% reported other ethnicity, and 15% had missing ethnicity data. The ethnic composition reflects the characteristics of the patient population at the screening clinic at the time of recruitment. The median age at diagnosis of breast cancer was 61 years, and the median period between initial blood donation and diagnosis was 2.3 years. Compared with controls, case subjects were more likely to report a prior benign breast condition (62.4% *versus* 51.2%; P = 0.005) and had a higher weight (mean, 69 *versus* 67 kg; P = 0.06) and Quetelet index (mean, 25.9 *versus* 25.5; P = 0.24). Breast cancer cases also had an earlier age of menarche (mean, 12 *versus* 13 years; P = 0.09) and a later age at first full-term pregnancy (mean, 26 *versus* 25 years; P = 0.04).

Table 2 LH status of breast cancer cases and controls, NYU Women's Health Study

LH status	Breast cancer cases (n = 270)	Controls (n = 540)	OR (95% CI)[a]	OR (95% CI)[b]
Normal LH (wild type)	239 (88.5%)	482 (89.3%)	1.00	1.00
Variant LH (heterozygotes)	29 (10.7%)	54 (10.0%)	1.08 (0.68–1.72)	1.11 (0.69–1.78)
Variant LH (homozygotes)	2 (0.7%)	4 (0.7%)	1.00 (0.18–5.50)	1.03 (0.18–5.71)
Variant LH (heterozygotes + homozygotes)	31 (11.5%)	58 (10.7%)	1.07 (0.68–1.69)	1.10 (0.70–1.74)

[a] Adjusted only for matching factors.
[b] Adjusted for height, Quetelet index, age at menarche, age at first full-term pregnancy (all as continuous variables), history of a prior benign breast condition (positive *versus* negative), and first-degree family history of breast cancer (positive *versus* negative).

Table 2 shows distribution of the variant LH among breast cancer cases and controls. Of 810 subjects included in the analysis, 89 had a low assay 1:assay 2 LH ratio (83 heterozygous and 6 homozygous subjects), corresponding to the variant LH prevalence rate of 11.0%. There was no significant difference in the frequency of variant LH between cases and controls (11.5% *versus* 10.7%, respectively; $P = 0.75$). Among cases, the median age of breast cancer diagnosis was similar in women with wild-type LH (61.4 years) and variant LH (60.2 years).

In logistic regression analyses, we computed ORs for breast cancer associated with LH status. The presence of the variant LH (heterozygotes plus homozygotes) was not associated with an apparent increase in breast cancer risk (OR, 1.07; 95% CI, 0.68–1.69). Adjustment for height, Quetelet index, age at menarche, age at first full-term pregnancy, history of a prior benign breast condition, and first-degree family history of breast cancer did not notably change the risk estimate (OR, 1.11; 95% CI, 0.69–1.78). Because frequency of the variant LH varies with ethnicity, we restricted analyses to Caucasian subjects, the most common ethnic group in the study, but the risk estimate remained close to unity (OR, 0.97; 95% CI, 0.53–1.77).

Discussion

This study was undertaken to determine whether the recently discovered variant LH, which is characterized by higher *in vitro* bioactivity in the stimulation of steroidogenesis (8, 9) and higher circulating levels of estradiol and testosterone (10) but a shorter circulatory half-life (8), is associated with breast cancer risk. It has previously been shown that the variant LH may be associated with clinical conditions including polycystic ovary syndrome (10, 11), menstrual disorders (13, 14), and delayed puberty (15) but not, to our knowledge, with breast cancer. In a cohort of mostly Caucasian women, we found no evidence that the variant LH genotype is associated with the risk of breast cancer diagnosed at age 50 years and older.

The major limitation of the study was its relatively small sample size, especially considering the low prevalence of the variant LH (heterozygous and homozygous) in our cohort (11%) as compared with previous observations in Scandinavia and Western Europe (4). As a result, the statistical power of the study was limited. Nevertheless, given our adjusted risk estimate of 1.11 (95% CI, 0.69–1.78), the upper confidence limit of 1.78 indicates that an increase in risk larger than 78% is unlikely, suggesting that the variant LH is not a major risk factor for breast cancer among women ages 50 years and older.

Only 6 of 810 subjects (2 cases and 4 controls) were homozygous for the variant LH. Although these numbers are small, a similar prevalence of homozygosity in cases and controls does not give substance to the argument that the effect of the variant LH on breast cancer is more pronounced in homozygous subjects than in heterozygous subjects.

With regard to the relationships between the variant LH and endogenous hormone levels, we had measured endogenous estrogens in postmenopausal women (16), but the previous publication included only a subset of the participants included in this study. We are currently in the process of performing laboratory analyses for endogenous steroid hormones among all breast cancer cases and controls in our cohort, and we plan to analyze the relationship between the variant LH and endogenous hormone levels.

In conclusion, the results of the present study do not appear to support the hypothesis that the variant form of LH is associated with an altered risk of breast cancer diagnosed at age 50 years and older. It is conceivable that the higher bioactivity of the variant LH, coupled with its shorter half-life, could compensate for each other with no apparent effect on breast cancer risk.

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